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SYNTHESIS OF N-α-BOC-N-ε-TRIBENZYL EDTA-L-LYSINE. AN AMINO ACID ANALOGUE SUITABLE FOR SOLID PHASE PEPTIDE SYNTHESIS

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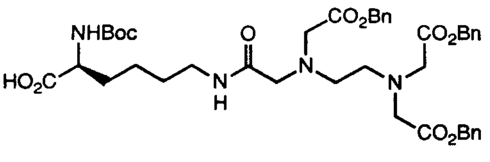
Abstract: The synthesis of an amino acid analogue suitable for appending ethylenediaminetetraacetic acid (EDTA) to internal amino acid residues of a peptide prepared from N-*tert*-butyloxycarbonyl- (Boc-) amino acids is described.

There has been considerable recent research directed towards the design and synthesis of protein- and nucleic acid-binding molecules bearing an appended ethylenediaminetetraacetic acid (EDTA) ligand. Attachment of EDTA to protein- or nucleic acid-binding molecules generates a class of compounds capable of affinity cleavage[1] of their protein[2,3] or DNA[1,4] target. Upon complexation with redox active metal ions such as Fe or Cu, these molecules generate a diffusible oxidant, presumably hydroxyl radical, which cleaves the DNA or protein backbone. The reaction is catalyzed by reducing agents such as dithiothreitol or sodium ascorbate and macromolecule cleavage occurs under physiologically relevant conditions of temperature, pH and salt concentration. If the protein- or nucleic acid-binding ligand is site-specific, cleavage is observed only at this site. Peptides carrying EDTA at the N-terminus or proximal to the C-terminus have been reported.[5] However, published methodology is inappropriate for the incorporation of EDTA at positions distant from the C-terminus of the peptide chain.[6] As part of a research program focused on affinity cleavage of proteins by peptide ligands, we sought a method for the placement of EDTA at any unique position, even one far from the C-terminus. Herein we report a straightforward, convergent synthesis of a modified lysine residue (1) suitable for the placement of EDTA *at any position in a peptide.*



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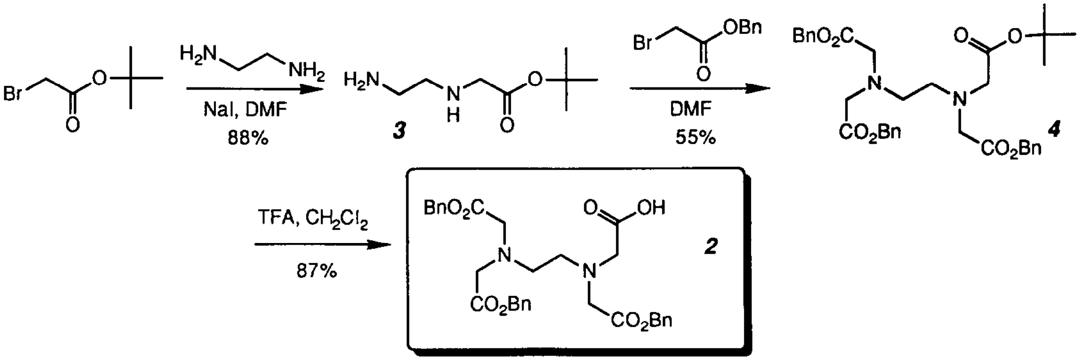
Compound **1** was designed to be compatible with Merrifield[7] solid-phase peptide synthesis employing N-*tert*-butyloxycarbonyl- (Boc-) protected amino acids. The three carboxylic acids of the EDTA portion are protected as benzyl esters, the same group used to protect aspartic and glutamic acid side chains. The α-amino group is protected with a Boc group. An intermediate in the synthesis of **1** is the tribenzyl ester of EDTA **2**, which is attached directly to the ε-amino group of lysine through an amide linkage. Compound **1** positions the EDTA functionality as close to the peptide backbone as possible. However, the convergent synthetic strategy permits spacers of varying length and structure to be placed between the EDTA and the α-carbon backbone and the construction of a family of closely related affinity cleaving peptides. The availability of these molecules should expand the scope and add flexibility in the design of affinity cleaving peptides directed against protein and nucleic acid targets.

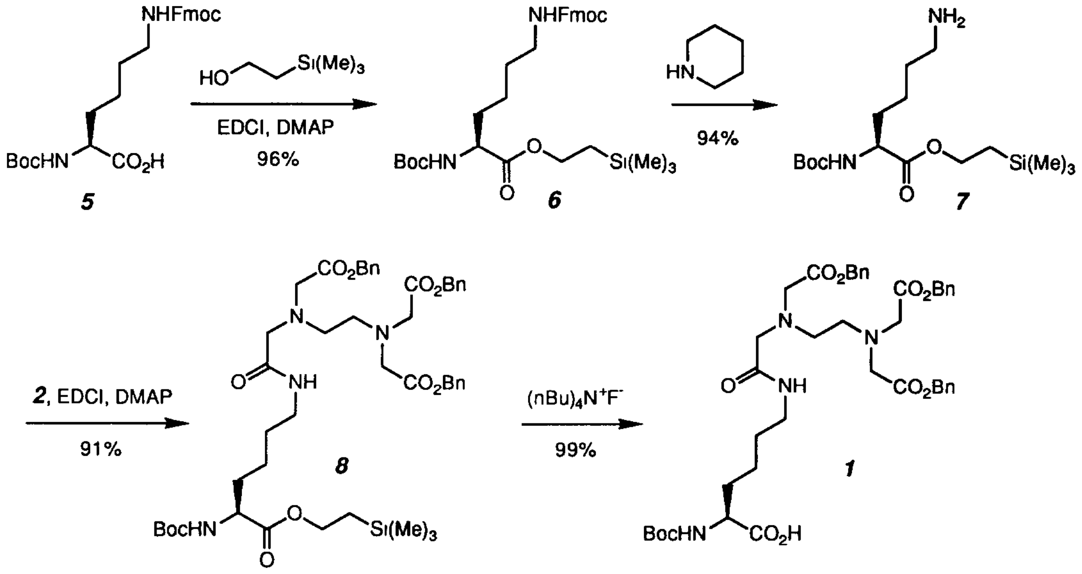
Scheme 1



The tribenzyl ester of EDTA **2** was synthesized in three steps as illustrated in Scheme 1. Commercially available *tert*-butyl-bromoacetate was reacted with excess ethylenediamine and sodium iodide to provide amine **3** in 88% yield.[8] Exhaustive alkylation[8] of **3** with benzyl bromoacetate provided a 55% yield of the mixed ester **4**. Compound **4** was transformed into EDTA tribenzyl ester **2** following a brief treatment with trifluoroacetic acid.[9] The overall yield of this sequence is 42% and the three steps can be performed on gram scale in 3-4 days.[10]

Our strategy for converting tribenzyl EDTA **2** into an amino acid suitable for Merrifield solid-phase peptide synthesis utilizes commercially available lysine derivative **5** and is shown in Scheme 2. Compound **5** is well-suited for our purposes because its ε-amino group is protected orthogonally as the 9-fluorenylmethyl carbamate (Fmoc).[11] Reaction of **5** with 2-(trimethylsilyl)ethanol provided the trimethylsilyl ester **6** in 96% yield.[12] Treatment with piperidine[13] removed the Fmoc group (94%) and generated amine **7**, which is in a form suitable for condensation with tribenzyl EDTA **1**. Carbodiimide catalyzed condensation of amine **7** with **2** occurred with 91% yield.[14] Finally, cleavage of the trimethylsilyl ethyl ester by use of tetrabutylammonium fluoride in THF generated **1** in an overall yield of 81% from **5**. Once incorporated into a peptide, the benzyl esters can be removed in the usual way.

Scheme 2



Summary

We have described a straightforward, convergent synthesis of an amino acid analogue (**1**) suitable for incorporation of EDTA at any prescribed residue in a synthetic peptide prepared using Boc-protected amino acids. Compound **1** is stable and is prepared easily on large scale. The ability to synthesize peptides carrying EDTA at any amino acid residue and at any distance from the α-carbon backbone will extend the scope of both protein and nucleic acid affinity cleaving experiments. Moreover, it introduces the intriguing prospect of using protein affinity cleavage to study the structures of partially folded peptide intermediates in solution[15] or conformational changes which occur in peptides and proteins upon ligand binding.

Experimental Section

General details: All reactions were performed in a nitrogen atmosphere. All reagents were reagent grade and were used without further purification unless noted otherwise. N-α-Boc-N-ε-Fmoc-L-Lys was purchased from Bachem Inc. Methylene chloride was distilled from calcium hydride. Dimethylformamide was purchased from Pierce Chemical Company and was Sequanal Grade. Diisopropylethylamine was distilled from potassium hydroxide and ethylenediamine was distilled and stored under nitrogen at 4°C. Thin layer chromatography (TLC) was performed on Silica Gel 60 F-254 precoated plates (250 µm, Merck). Flash chromatography[16] was performed using Merck silica gel (Silica gel 60, 230-400 Mesh). Proton nuclear magnetic resonance spectra were recorded with Bruker WM 250 or AM 500 MHz instruments and are reported in parts per million (ppm) downfield from Me_4Si. Coupling constants are reported in Hertz (Hz). Infared spectra were recorded from films on KBr plates using a Nicolet FT-IR 5-SX spectrophotometer. Low resolution mass spectra were obtained with a HP 5985 GS-MS and high resolution spectra were obtained with a Kratos MS 80RFA. Optical rotations were obtained with a Perkin-Elmer 241 Polarimeter.

***Tert*-butyl ester amine 3**: To a solution of sodium iodide (558 mg, 3.72 mmol, 1 equiv.), ethylenediamine (4.44 g, 74.0 mmol, 20 equiv.) and 500 µL DMF at 4°C was added 726 mg (3.72 mmol, 1 equiv.) of *tert*-butyl bromoacetate over 30 minutes. The solution was stirred for an additional hour, then placed under high vacuum (1.5 torr) to remove DMF and excess diamine. Flash chromatography on SiO_2 using 50% methanol in CH_2Cl_2 as the eluent provided 570 mg (88%) of **3** as a colorless oil. *^1H NMR* (250 MHz, CD_3OD) δ 3.33 (s, 2H, $NHCH_2CO$), 2.94 (dist t, 2H, J=5.9, $NHCH_2CH_2NH_2$), 2.84 (dist t, 2H, J=5.9, $NHCH_2CH_2NH_2$), 1.47 (s, 9H, $C(CH_3)_3$). *IR* (KBr, film) ν 3343, 3269, 2981, 1736 cm^{-1}. *MS* (EI) calculated for $C_8H_{18}N_2O_2$ 174.1, observed 174.1. *TLC* (SiO_2, 100% ammonia saturated methanol) R_f 0.50.

EDTA-tribenzyl-*tert*-butyl ester 4: To a solution of 90 mg (0.52 mmol, 3 equiv.) **3** and 214 mg diisopropylethylamine (1.66 mmol, 3.2 equiv.) in 2 mL DMF at 4 °C was added 380 mg (1.66 mmol, 3.2 equiv.) benzyl bromoacetate. The reaction was stirred for 30 minutes at 4°C, then for 22 hours at 45 °C. The solvent was removed under high vacuum, and the residue redissolved in 5 mL CH_2Cl_2 and washed sequentially with 5 mL $NaHCO_3$(sat), 5 mL NaCl(sat) and 5 mL water. The organic layer was dried (Na_2SO_4) and the solvent removed under reduced pressure. The residues were chromatographed on SiO_2 using 20% ethyl acetate in hexane as eluent to give 177 mg (55%) of **4** as a colorless oil. *^1H NMR* (250 MHz, $CDCl_3$) δ 7.31 (m, 15H, aromatic), 5.08

(s, 6H, CH_2Ph), 3.63 (s, 4H, $N(CH_2CO_2Bn)_2$), 3.59 (s, 2H, CH_2CO_2Bn), 3.42 (s, 2H, $CH_2CO_2C(CH_3)_3$), 2.87 (bs, 4H, NCH_2CH_2N), 1.41 (s, 9H, $C(CH_3)_3$). *IR* (KBr, film) v 2973 , 1733, 1367, 740, 696 cm^{-1}. *HRMS* (CI) calculated M+H for $C_{35}H_{43}N_2O_8$ 619.3019, observed M+H 619.3021. *TLC* (SiO_2, 30% ethyl acetate in hexanes) Rf 0.33.

Tribenzyl EDTA 2: To a solution of 150 mg (0.24 mmol) **4** in 500 μL CH_2Cl_2 was added 1 mL of trifluoroacetic acid. The reaction was stirred for 1 hour at ambient temperature, at which time TLC analysis showed complete consumption of **4**. The solvent was removed under reduced pressure and the residues dissolved in 5 mL CH_2Cl_2 and washed sequentially with 5 mL $NaHCO_3$(sat), 5 mL NaCl(sat), and 5 mL water. The organic phase was dried (Na_2SO_4) and the solvent removed under reduced pressure to provide 118 mg (87%) of **2** as a pale green oil. *^1H NMR* (250 MHz, DMSO+TFA) δ 7.42 (m, 15H, aromatic), 5.19 (s, 2H, OCH_2Ph), 5.11 (s, 4H, $N(CH_2CO_2CH_2Ph)_2$), 4.25 (s, 2H, NCH_2CO_2H), 4.10 (s, 2H, NCH_2CO_2Bn), 3.83 (s, 4H, $N(CH_2\ CO_2Bn)_2$), 3.35 (bm, 2H, NCH_2CH_2N), 3.16 (bm, 2H, NCH_2CH_2N). *IR* (KBr, film) v 3307, 2953, 1760, 1600, 1455, 1403, 1195, 1131, 1007, 736, 696 cm^{-1}. *HRMS* (FAB) calculated M+H for $C_{31}H_{35}N_2O_8$ 563.2393, observed M+H 563.2394. *TLC* (SiO_2, 10% methanol, 0.8% water in CH_2Cl_2) Rf 0.35.

N-α-Boc-N-ε-Fmoc-L-Lys-O-Tmse ester 6: To a solution of 920 mg (1.96 mmol, 1 equiv.) N-α-Boc-N-ε-Fmoc-L-Lys, 753 mg (3.92 mmol, 2 equiv.) 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and a catalytic amount of 4-dimethylaminopyridine (DMAP) in 10 mL CH_2Cl_2 was added 347 mg (2.94 mmol, 1.5 equiv.) 2-(trimethylsilyl)-ethanol. After stirring 12 hours at room temperature, the reaction mixture was diluted with 40 mL CH_2Cl_2 and washed successively with 50 mL $NaHCO_3$(sat), 50 mL NaCl(sat) and 50 mL water. The organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. The residues were chromatographed on SiO_2 using 30% ethyl acetate in hexane as the eluent to give 1.07 g (96%) of **6** as a colorless oil, $[\alpha]_D^{25}$ -12° (c 1, CH_3OH). *^1H NMR* (250 MHz, CD_3OD) δ 7.78 (d, J=7.3, 2H, aromatic), 7.62 (d, J=7.2, 2H, aromatic), 7.33 (m, 4H, aromatic), 4.34 (d, J=6.6, 2H, $CHCH_2O$), 4.18 (m, 3H, $CHCH_2O$ and OCH_2CH_2TMS), 4.03 (dd, J=4.9, 8.9, 1H, αCH), 3.09 (t, J=4.3, 2H, $NHCH_2CH_2$), 1.41 (s, 9H, $OC(CH_3)_3$), 1.2-1.7 (m, 6H, $(CH_2)_3$), 1.00 (dist t, J= 4.1, 2H, $TMSCH_2CH_2$), 0.02 (s, 9H, TMS). Selected decoupling (250 MHz, CD_3OD): Irradiation at δ 0.99, simplification at δ 4.18. Irradiation at δ 4.18, simplification at δ 4.34 and 0.99. *IR* (KBr, film) v 3340, 2952, 1735-1690, 1250, 1169, 1045, 858, 837, 758, 740 cm^{-1}. *HRMS* (FAB) calculated M+H for $C_{31}H_{45}N_2O_6Si$ 569.3047, observed M+H 569.3044. *TLC* (SiO_2, 30% ethyl acetate in hexanes) Rf 0.33.

N-α-Boc-L-Lys-O-Tmse ester 7: Compound **6** (558 mg, 0.98 mmol) was dissolved in 5 mL piperidine and stirred at ambient temperature. After 2 hours, the piperidine was removed under vacuum and the residues chromatographed on SiO_2 using 10% ammonia-saturated methanol in CH_2Cl_2 as the eluent to give 320 mg (94%) of **7** as a colorless oil, $[\alpha]_D^{25}$ -10° (c 1, CH_3OH). *^1H NMR* (250 MHz, CD_3OD) δ 4.20 (dist t, J=8.1, 2H, OCH_2CH_2TMS), 4.04 (dd, J=4.9, 8.8, 1H, aC**H**), 2.62 (t, J=7.0, 2H, CH_2**CH_2**NH_2), 1.2-1.7 (m, 6H, (C**H**$_2$)$_3$$CH_2NH_2$), 1.43 (s, 9H, C(C**H**$_3$)$_3$), 1.01 (dist t, J=8.4, 2H, OCH_2**CH_2**TMS), 0.05 (s, 9H, TMS). *IR* (KBr, film) ν 3367, 2951, 1736, 1715, 1250, 1166, 1047, 861, 837 cm^{-1}. *HRMS* (FAB) calculated M+H for $C_{16}H_{35}N_2O_4Si$ 347.2366, observed M+H 347.2367. *TLC* (SiO_2, 10% ammonia saturated MeOH in CH_2Cl_2) Rf 0.26.

N-α-Boc-N-ε-EDTA(Bn)$_3$-L-Lys-O-Tmse ester 8: A solution of **7** (39.0 mg, 113 μmol, 1 equiv.), **2** (96 mg, 170 μmol, 1.5 equiv.), EDCI (43.4 mg, 226 μmol, 2 equiv.) and DMAP (catalytic amount) in 1 mL CH_2Cl_2 was stirred at room temperature for 23 hours. The solution was diluted to 5 mL with CH_2Cl_2 and washed successively with 5 mL 10% citric acid, 5 mL $NaHCO_3$(sat), 5 mL NaCl(sat), and 5 mL H_2O. The organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. The residues were chromatographed on SiO_2 using 8% methanol in CH_2Cl_2 as the eluent to give 91.3 mg (91%) of **8** as a pale yellow oil, $[\alpha]_D^{25}$ -8° (c 1, CH_3OH). *^1H NMR* (250MHz, CD_3OD) δ 7.33 (m, 15H, aromatic), 5.13 (s, 6H, PhC**H**$_2$CO), 4.10 (dist t, J=8.5, 2H, OC**H**$_2$$CH_2TMS$), 4.06 (dd, J=5.1, 8.8, 1H, aCH), 3.58 (s, 4H, N(C**H**$_2$$CO_2Bn)_2$), 3.44 (s, 2H, NC**H**$_2$CONH), 3.26 (s, 2H, NC**H**$_2$$CO_2Bn$), 3.14 (t, J=6.7, 2H, CONHC**H**$_2$), 2.75 (m, 4H, NC**H**$_2$C**H**$_2$N), 1.3-1.7 (m, 6H, $(CH_2)_3$), 1.40 (s, 9H, $OC(CH_3)_3$), 0.96 (dist t, J=8.5, 2H, TMSC**H**$_2$), 0.02 (s, 9H, TMS). *IR* (KBr, film) ν 3305, 3066, 3033, 2949, 1740, 1715, 1663, 861, 835 cm^{-1}. *HRMS* (FAB) calculated M+H for $C_{47}H_{67}N_4O_{11}Si$ 891.4578, observed M+H 891.4603. *TLC* (SiO_2, 10% methanol in CH_2Cl_2) Rf 0.52.

N-α-Boc-N-ε-EDTA(Bn)$_3$-L-Lys 1: To a solution of 29.4 mg (34 μmol) **8** in 500 μL THF was added 200 μL of a 1 M solution of tetrabutylammonium fluoride in THF. After three minutes, the solution was cooled to 4°C, diluted with 500 μL H_2O and evaporated under reduced pressure. The residues were dissolved in 5 mL ethyl acetate and washed with 5 mL 10% citric acid and two 5 mL portions of water, dried (Na_2SO_4) and the solvent removed under reduced pressure. The residues were dried *in vacuo* over P_2O_5 to provide 26.6 mg (99%) of **1** as a colorless oil, $[\alpha]_D^{25}$ -4° (c 1, CH_3OH). *^1H NMR* (250 MHz, d_6-acetone + D_2O) δ 7.35 (m, 15H, aromatic), 5.11 (s, 6H, PhC**H**$_2$O), 4.10 (dd, J=4.9, 8.5, 1H, aCH), 3.68 (s, 4H, N(C**H**$_2$$CO_2Bn)_2$), 3.68 (t, 2H, CONHC**H**$_2$$CH_2$), 3.48 (s, 4H, COC**H**$_2$N), 2.96 (t, J=6.2 , 2H, NC**H**$_2$$CH_2N$), 2.65 (t, J=6.2 , 2H, NCH_2C**H**$_2$N), 1.2-1.7 (m, 6H, $(CH_2)_3$), 1.39 (s, 9H, $OC(CH_3)_3$); Selected decoupling (250 MHz, d_6-acetone + D_2O) Decoupling at δ 1.50, simplification at δ 3.68. *^{13}C NMR* (63 MHz, d_6-acetone) δ 174.1, 171.7, 171.6, 170.83, 137.4, 137.3, 129.34, 129.30, 128.92, 128.87, 128.81, 127.5, 127.4, 79.2, 66.6, 66.5, 66.4, 64.6, 57.1, 55.9, 55.7, 54.7, 54.1, 52.9, 51.6, 38.9, 32.2, 28.6, 28.5, 28.4, 28.2, 23.8. *IR* (KBr, film) v 3395, 3091, 3069, 3033, 2954, 2933, 2868, 2510, 1950, 1750-1668, 1497, 1455, 1367, 1303, 1240, 1172, 1002, 739, 698. *HRMS* (FAB) calculated M+H for $C_{42}H_{55}N_4O_{11}$ 791.3867, observed M+H 791.3820. *TLC* (SiO_2, 10% methanol, 0.8% water in CH_2Cl_2) R_f 0.32.

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10. We also attempted to prepare **2** by partial esterification of EDTA with benzyl alcohol, as recently described for the cyclohexyl derivative by Dervan and coworkers.[6] This reaction provided **2** in 3% yield.
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